QUANTITATIVE IMAGING MASS SPECTROMETRY OF ENDOGENOUS METABOLITES USING IN-LINE INTERNAL STANDARDS AND STABLE ISOTOPE LABELED MIMETIC MODEL

Bindesh Shrestha,[1] Khaja Muneeruddin,[2] Scott A. Shaffer[2]

[1] Waters Corporation, Beverly, MA 1Department of Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School

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INTRODUCTION

- Quantitative spatial distributions of small molecules, such as metabolites and drugs, are vital for studying any spatial metabolic processes
- Mass spectrometry (MS) imaging, such as matrix-assisted laser desorption ionization (MALDI) and desorption Electrospray Ionization (DESI), can map the spatial distribution of metabolites
- DESI often provide a complementary metabolomic coverage, allowing for quantitative mapping of compounds typically inaccessible in MALDI imaging
- Here, we describe a workflow of DESI-MS imaging to obtain a quantitative information of endogenous metabolites and drug using mimetic models and in-line internal standard reference
- In the workflow, tissue memetic models are spiked with varying concentration range of metabolites or drugs of interest, an in-line internal standard reference was added to DESI sprayer solvent to normalize and measure the relative concentration of those molecules between two sets of tissue measurements

DESI (DESORPTION ELECTROSPRAY IONIZATION)

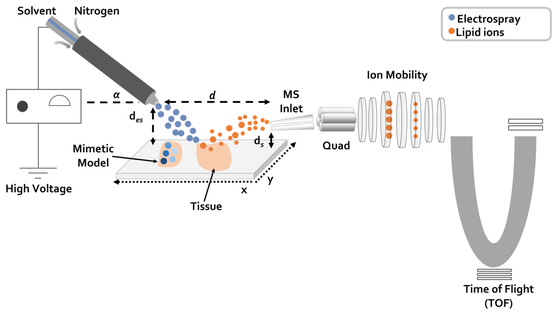

Figure 1. *Schematic representation of desorption electrospray ionization (DESI) mass spectrometry imaging ion source coupled with quadrupole time-of-flight (ToF) mass spectrometer equipped with ion mobility separation*

In DESI, a pneumatically assisted (nitrogen gas) charged electrospray jet is focused onto a sample surface , such as tissue section, to desorb analytes. The ionized analytes are carried into a mass spectrometer inlet and transferred for detection. The sample stage is moved in a two plane dimension to obtain a pixel-by-pixel mass spectrometric profiles. The ion intensities of a selected ion(s) at the each pixel is plotted in a xy dimension to obtained its false color image.

METHODS

- Animal tissues were flash-frozen in liquid nitrogen before cryo-sectioning, freshly excised sections were thaw mounted on glass slides and stored in - 80 °C until use
- For mimetic models, standard was added to a similar adjoining tissue sections at various concentration range to create calibration curve
- An in-line internal standard reference, such as LeuEnk, was added in DESI sprayer solvent to be used as a lock-mass for correcting any potential *m/z* drift
- A qToF mass spectrometer (SYNAPT HDMS G2-S*i*, Waters Corporation, Milford, MA) equipped with DESI imaging ion source was used to collect the data
- MS imaging data was processed, and analyzed using High Definition Imaging (HDI) 1.5 software with MassLynx 4.2 was used as data acquisition control (Waters Corporation).

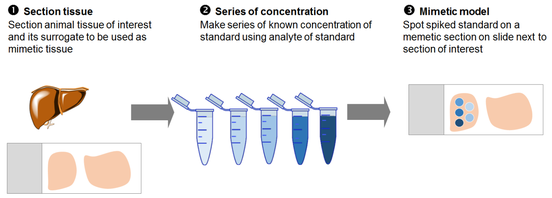

Figure 2. *Schematic representation of sample preparation used for quantitation of the drug molecule used in this study is shown here. Briefly, various concentrations of the drug was spotted on a control tissue to be used as calibration points. For exogenous molecules such as drugs, standard was spotted and for endogenous molecules, stable isotope labeled molecule can be spotted.*

DESI MASS SPEC IMAGING

Ion source: DESI 2D stage (Waters Corporation, Milford, MA)

Mass Spectrometer: SYNAPT G2-S*i* HDMS qToF (Waters Corporations, Milford MA)

DESI Conditions:

- 98% methanol at 3 µL/min
- Nebulizing gas pressure of 0.5 MPa nitrogen
- 3.0 kV sprayer voltage
- Positive polarity
- Mass range 50 -1,200 m/z in resolution mode of SYNAPT G2-Si

Data Management:

- Acquisition: MS Data acquisition was setup in High Definition Imaging (HDI) version 1.4 and acquired using MassLynx4.2
- Processing: RAW files was processed in HDI 1.5
- Analysis: MS imaging data visualization and regions of interest (ROI) generation was done using HDI 1.5

QUANTIATION OF EXOGENEOUS DRUG

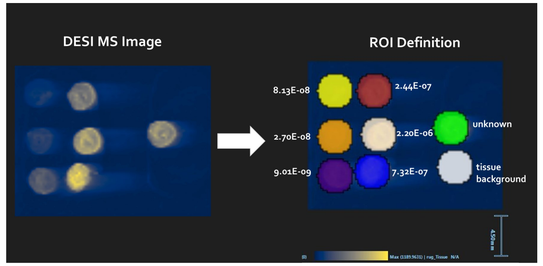

Figure 3. *Regions of interest or ROI were defined on the spotted drug (verapamil) . Each spot represents an unique drug concentration. The ROI was exported to RAW MassLynx file or peak list with normalized intensities. ROI of mock unknown and background was also obtained.*

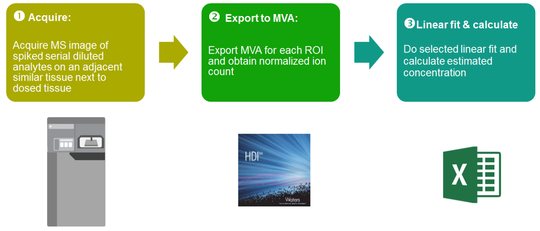

Figure 4. *The workflow illustrates calculation of the intensities of an unknown. Briefly, total MS response of each concentration was measured by DESI imaging of spots. The intensities were exported as separate ROIs. The measured intensities was used to plot a calibration curve and measure concentration of unknown*

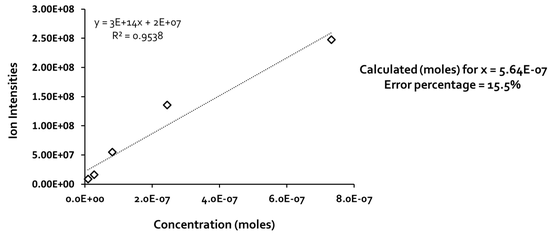

Figure 5. *The peak intensity of ROI corresponding each concentration was exported as an individual mass spectrum. The ion intestines of analyte standard for each plot was plotted and fitted with linear trendline. Based on the equation, unknown concentration for X was calculated. The error was calculated based on real known concentration of the X .*

METABOLITE IMAGING

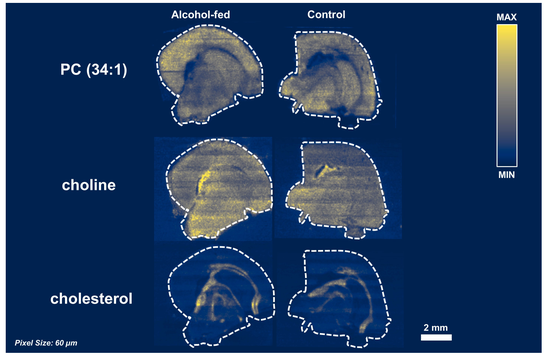

Figure 6. *DESI images of alcohol-fed mice brain and its control counterpart is shown. The MS images were normalized to a stable isotope-label internal standard (d9-choline) and plotted on identical heatmap scale. The ROI analysis after the normalization with the internal standard showed that choline and cholesterol were 1.3 and 1.4 times more abundant in alcohol-fed brain, respectively. The PC peak was at the same level in both brain.*

CONCLUSIONS AND FUTURE DIRECTIONS

- DESI MS imaging allows for quantitative measurements for molecules in tissue typically inaccessible by using other imaging techniques such as MALDI
- Preliminary data illustrates quantitative imaging of small molecules such as metabolites and drugs can be done using DESI
- Future work would include utilization of mimetic model consists of either homogenate spots or band of a control tissue spiked with a range of concentrations of stable isotope labeled metabolite (see figure 7)

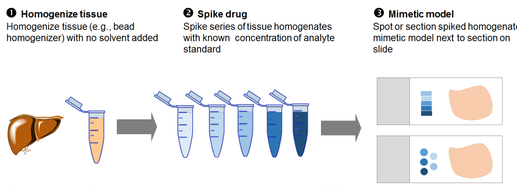

Figure 7. *Workflow for future experiments with quantitative DESI imaging using mimetic model of either spiked homogenate spots or section band. This mimetic model ensures a similar degree of analyte extraction and ion suppression as analytes.*

REFERENCES

[1] M. Reid Groseclose, Stephen Castellino, *"A Mimetic Tissue Model for the Quantification of Drug Distributions by MALDI Imaging Mass Spectrometry"* Anal. Chem., **2013**, 85, 21, 10099-10106

[2] Jeremy A. Barry, M. Reid Groseclose, Donna D. Fraser, Stephen Castellino. *"Revised Preparation of a Mimetic Tissue Model for Quantitative Imaging Mass Spectrometry"*, 24 August 2018, PROTOCOL (Version 1) available at Protocol Exchange https://doi.org/10.1038/protex.2018.104

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